GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

No action
4 OK

Date: 2/1/79 OK

Project Title: Porphyrin Ions and Radicals in Metabolism: Physical Properties

Project No: G-33-M01 (Continuation of G-33-630)

Project Director: Dr. R. H. Felton Green card

Sponsor: DHEW/PHS/NIH - National Institute of Arthritis, Metabolism & Digestive Diseases

Agreement Period: From 1/1/79 Until 12/31/79 (10 Year)

Type Agreement: Grant No. 2 R01 AM14344-10

Amount: $107,292 New PHS Funds (G-33-M01)
6,504 GIT Contribution (G-33-335)
$113,796 Total

Reports Required: Annual Progress Reports with Continuation Applications
Terminal Progress Report upon Grant expiration

Sponsor Contact Person (s):

Technical Matters

Robert Katz, PH.D.
Chief, Metabolic Diseases Section, EP
National Institute of Arthritis, Metabolism,
& Digestive Diseases
Bethesda, MD 20014

Contractual Matters
(thru OCA)

Linden F. Neff
Grants Management Officer, EP
National Institute of Arthritis,
Metabolism, & Digestive Diseases
Bethesda, MD 20014

Defense Priority Rating: None

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director--EES
Accounting Office
Procurement Office
Security Coordinator (OCA)
Reports Coordinator (OCA)

Library, Technical Reports Section
EES Information Office
EES Reports & Procedures
Project File (OCA)
Project Code (GTRI)
Other

CA-3 (3/78)

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SR 1849

SPONSORED PROJECT TERMINATION SHEET

Date August 30, 1983

Project Title: Porphyrin Ions and Radicals in Metabolism: Physical Properties

Project No: G-33-M01

Project Director: Dr. R. H. Felton

Sponsor: National Institute of Arthritis, Metabolism and Digestive Diseases

Effective Termination Date: 12/31/79

Clearance of Accounting Charges: 3/31/80

Grant/Contract Closeout Actions Remaining:

- ☐ ~~Final Invoice and Closing Documents~~ ROE prepared 10/7/81
- ☐ Final Fiscal Report
- ☒ Final Report of Inventions
- ☐ Govt. Property Inventory & Related Certificate
- ☐ Classified Material Certificate
- ☐ Other _____

Assigned to: Chemistry (School/~~Laboratory~~)

COPIES TO:

Administrative Coordinator	Research Security Services	EES Public Relations (2)
Research Property Management	Reports Coordinator (OCA) ✓	Computer Input
Accounting	Legal Services (OCA)	Project File
Procurement/EES Supply Services	Library	Other _____

FORM OCA 10-78

Final Report

G-33-M01

SECTION IV

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →	GRANT NUMBER	
SECTION IV—SUMMARY PROGRESS REPORT	AM14344-10	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial) Felton, Ronald H.	PERIOD COVERED BY THIS REPORT FROM	THROUGH
NAME OF ORGANIZATION Georgia Institute of Technology	01/01/79	09/15/79

TITLE (Repeat title shown in Item 1 on first page)
Porphyrin Ions in Metabolism: Physical Properties

1. List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
2. List all additions and deletions in professional personnel and any changes in effort.
3. Progress Report. (See Instructions)

1a. Publications

R. H. Felton, L. D. Cheung, R. S. Phillips, and S. W. May, "A Resonance Raman Study of Substrate and Inhibitor Binding to Protocatechuate-3,4-Dioxygenase, Biochem. Biophys. Res. Commun., 85, 844-40 (1978).

L. D. Cheung, N.-T. Yu, and R. H. Felton, "Resonance Raman Spectra and Excitation Profiles of Soret-Excited Metalloporphyrins," Chem. Phys. Lett. 55, 527 (1978).

R. H. Felton and N.-T. Yu, in "The Porphyrins," ed. D. Dolphin, Vol. III, Chapter 8, p. 347-393 (1978), Academic Press.

R. H. Felton, in "The Porphyrins," ed. D. Dolphin Vol. V, Chapter 3, p. 53-125 (1978), Academic Press.

A. Lanir, N.-T. Yu, R. H. Felton "Conformational Transition and Vibronic Couplings in Acid Ferricytochrome c: A Resonance Raman Study", Biochemistry, 18, 1656 (1979).

H. Sambe and R. H. Felton, "A Semi-empirical Xα Calculation of Porphine", Chem. Phys. Lett., 61, 69 (1979).

1b. Publications in Press

W. L. Barrow, H. Sambe, and R. H. Felton, "Semi-empirical Xα Calculations in the Vibronic Framework. $C_2H_4^+$ and H_2O^+ Geometries", Chem. Phys. Lett.

R. H. Felton, L. D. Cheung, R. S. Phillips, and S. W. May, "Resonance Raman Spectra of Protocatechuate-3,4-Dioxygenase Theory of the Charge-Transfer Enhancement," J. Am. Chem. Soc.

2. Personnel Changes

Deleted	Added
Dr. L. D. Cheung	Mr. W. L. Barrow Dr. Y. Harel

3. Progress Report - see attached pages.

3. Progress Report: 01/01/78 - 09/15/79

1. Objectives

1. Utilization of resonance Raman spectroscopy (RRS) to elucidate active site structure in metalloenzymes.
2. Investigation of the mechanism of charge-transfer enhancement in RRS.
3. Synthesis of model porphyrin compounds for the purpose of simulating the heme environment in peroxidase.
4. Use of the semi-empirical Xα theory in item 2, and its extension to organometallic compounds.
5. Mechanism of electron transfer in porphyrins.

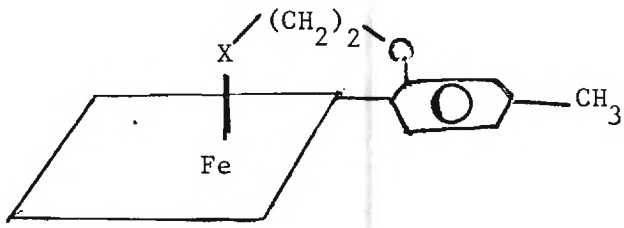
2. Progress Report

Resonance Raman (RR) spectra of high-spin acid cytochrome c was studied over the pH range 1.5-4.0. Using the structure-sensitive, anomalously polarized band at ca. 1590 cm^{-1} and the expanded core model, we suggest that acid cytochrome c is an example of in-plane, high-spin iron(III) porphyrins with weak-field axial ligands. In the present case displacement of protonated His and Met by water is likely. Protocatechuate-3,4-dioxygenase carries out the enzymatic oxygenation of protocatechuate acid to form β-carboxy-cis,cis-muconic acid. The RR data are clearly interpretable in terms of tyrosine coordination of ferric ion. Addition of inhibitors, 3-fluoro-4-hydroxybenzoate or 3-chloro-4-hydroxybenzoate provide spectra with new bands at 1303 and 653, or 1592, 1487, 1299, and 643 cm^{-1}, respectively. Sepctral studies of model Fe(III)-phenolate complexes confirms assignments of the new lines to inhibitor binding at the iron. Comparison of the inhibitor intensity ratio to Tyr scattering intensity suggests that the active site has 2 Tyr. The high-frequency region (700-1600 cm^{-1}) is similar in transferrin and PCD, but in the latter compound low-frequency bands at 593, 521, and 274 cm^{-1} are not apparent in transferrin. One of the pair at 500-600 cm^{-1} is assigned to Tyr, and the 274 cm^{-1} peak may be due to an Fe-S vibration. This low-frequency peak dissappears in enzyme-inhibitor or enzyme-substrate RR spectra. Thus, RR spectra for the first time have provided evidence during binding for specific modification of the inner ligation sphere of iron.

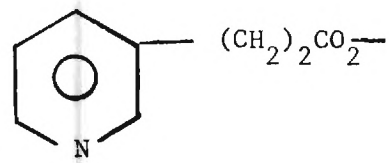
The mechanism of RR enhancement in charge-transfer electronic excitation depends upon the change in geometry during the excitation. We show that a vibronic picture provides an easily interpretable view in which the strength of RR intensities are governed by the coupling between the s^{th} normal coordinate, Q_s, and the i^{th} donor orbital with energy ε_i to the j^{th} acceptor orbital, this coupling is $\chi_s = \frac{1}{\sqrt{2}} \frac{\partial}{\partial Q_s} [\varepsilon_j - \varepsilon_i]$. The Xα theory (vide infra) provides $\partial\varepsilon_i/\partial Q_s$ and explains why certain phenolate modes are seen in PCD and transferrin RR spectra. The remaining term, $\partial\varepsilon_j/\partial Q_s$, is the change in the metal orbital energy (assumed to be the acceptor atom) with motion of nearest neighbor atoms described by Q_s. If this term is large, as it might be when Q_s contains a metal-ligand

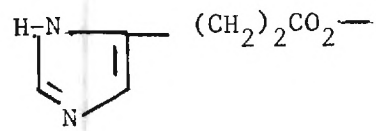
strecth, then low-frequency modes characteristic of the metal will appear. Most interesting, is the case when Q_s is a mode containing a metal-other ligand stretch. It is possible to envisage situations in which RR spectra of charge-transfer transitions will exhibit this mode, even though the ligand is not involved in the transition. Perhaps one of the pair at 593, 521 cm^{-1} in PCD is characteristic of an Fe-O or Fe-N stretch with a ligand distinct from Tyr.

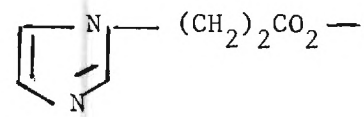
In a continuing effort to understand the chemistry, electronic structure, and biological occurrence of higher oxidation states of iron porphyrins, we have synthesized the following porphyrins bearing linked coordinating groups:



Tetra -(p-tolyl)porphine

I : X = 

II : X = 

III : X = 

In CH_2Cl_2, the polarographic behavior of I-III is summarized in the Table

Table I. Polarographic Data (vs. sce (aq.)

Compound	$\varepsilon_{1/2}^{ox}$ (2)	$\varepsilon_{1/2}^{(ox)}$(1)	$\varepsilon_{1/2}^{(red)}$(1)	$\varepsilon_{1/2}^{(red)}$(2)
I	1.10V	0.90	-0.66	0.85
II	0.97	0.69	-0.28	-
III	0.88	0.69	-0.20	-

The pyridine complex (I) exhibits reversible behavior at all waves. Controlled potential electrolysis at $\varepsilon_{1/2}^{ox}$ (1) yields a compound, Fe(IV), whose optical spectrum is similar to that reported for $Fe(IV)TPP^+Cl^-$. Both imidazole-linked porphyrins II, III exhibit irreversible polarographic behavior (0.5V - 100V/sec sweep) at the 0.79V wave. At the present, we believe that imidazole is oxidized at $\varepsilon_{1/2}^{ox}$(1). Interestingly, oxidized imidazole does not react with solvent, rather electron-transfer is rendered slow during the reductive sweep, since the Fe(III) →Fe(II) wave is recovered intact in II, III, and no degradation or loss of compound at the electrodes is observed with multiple sweep cyclic voltammetry. Tentatively, we believe that Im retains the extra oxidizing equivalent. In view of the current suggestions that Compounds II of horseradish peroxidase and cytochrome c peroxidase contain Fe(IV), the chemistry of II and III is surprising.

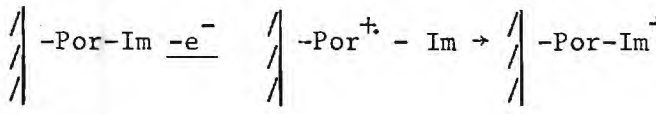
The optical spectra of the linked Fe(III) porphyrins show a moderately intense band at 325-330 nm, which disappears upon oxidation or reduction of I. This band is assigned to the Fe(III)←py, Im charge transfer and is an excellent candidate for resonance Raman spectroscopy.

The semi-empirical Xα theory provides valuable insight into the mechanism of RR enhancement in charge-transfer complexes. The program also provides relative geometry shifts of excited and ground electronic states. Our investigations with Xα electronic structure are aimed toward incorporation of transition metals, but we have already shown its utility in interpreting photoelectron data of metal-free porphyrins, and in predicting ionic geometry of ethylene and water.

A Z-80 microcomputer is now being interfaced to the Raman spectrometer. With this addition, data collection of weak pulse or cw-excited spectra will be possible. Additionally, the dual-channel counter in conjunction with a partitioned, rotating cell will provide a method to collect Raman difference spectra; this technique will be extremely useful in measuring small shifts between isotopic species. Some equipment for nanosecond flash spectroscopy has been purchased. One major change in design replaces oscillographic capture of the transient by a Tekronix Signal Digitizer ($14,500). The digitizer may be interfaced nicely to the Z-80 microcomputer and provides the capability of signal averaging of transients. Thus low-intensity (N_2 laser) pumping flashes, which cause only a small change in optical density, can be used.

3. Research Goals for the Period 01/01/80 - 12/31/80

A major effort is planned to characterize the redox chemistry and electron-transfer process in heme-linked imidazole and species in which other ions are substituted for iron. We know already that metal-free linked imidazole porphyrin exhibits irreversible oxidation waves, whereas imidazole itself is not oxidized at this potential (1.1 volt). The simplest explanation of the voltammetric results is that the sequence:



Pt electrode

takes place, and the return is irreversible. If the porphyrin acts as an electron-transfer agent, then some obvious questions are raised: (1) can we prove imidazole oxidation by chemical trapping with nucleophiles, (2) by varying the metal in the series Mg, Zn, Co at what potential is Im oxidation occurring, (3) if the porphyrin moeity is changed from TPP to OEP, will this alteration affect the potential of Im oxidation, (4) will electrodes of different composition, e.g. Ag or carbon influence the catalytic decomposition of Im, (5) how are rates of H_2O_2 decomposition or reaction influenced? The goal of preparing stable, low-spin iron(IV) porphyrin will be pursued with the pyridine complex, I.

For RR spectral studies, we have arranged with Dr. Nick Serpone to obtain Os(IV)OEP(L_1)(L_2) complexes. These complexes have quite different optical spectra as a function of L_1, L_2, and we are interested if (1) an oxidation state marker band is found in Fe(II) and Fe(III) porphyrins at 1374-1360 cm^{-1}; is it operative as a measure of $d_\pi - L_\pi$ electronic bonding in the Os complexes, and (2) a number of additional transitions are seen in the near uv (Soret) region; can these be assigned in terms of charge-transfer excitations? The linked porphyrins (I-III) provide excellent cases for examination of the enhancement of py or Im modes with excitation into the 325 nm band of the Fe(III) species. Will the excitation profiles behave as we found with PCD, i.e. peaking to red of λ_{max}?

The question of Fe(IV) existence is not challenged in HRP II or cyt c II; however, the evidence hinges upon Mossbauer studies. A more direct method is edge absorption of X-rays. In colloboration with Bill Parson., we have begun an EXAFS study of model Fe(IV) porphyrins and HRP II. An overall shift by ca. 2eV of the edge absorption to higher energies would provide good evidence for Fe(IV) in these systems. If the spectra taken in a fluorescence excitation mode are high-quality, then EXAFS might yield bond length changes and nearest neighbor distances in the peroxidase.

We have developed a new approach to minimal basis electronic calculations of organometallic compounds containing transition metals. The problem is that incorporation of atomic 4s and 4p orbitals in addition to 3d metal atomic orbitals is incorrect in a minimal basis theory, e.g. CNDO or extended Huckel theory. Only by requiring orthogonality to the metal ion core and incorporating the core explicitly in the formalism can we generate the proper behavior of ligand

orbitals near the metal core. The novel approach we are implementing is to use only 3d metal orbitals and the metal core, and then generate the higher metal + ligand molecular orbitals from the ligand orbitals. All formal development is carried out at the Xα level. We hope to generate $\partial\varepsilon_i/\partial Q_s$ for metal-ligand vibrations and thus interpret low-frequency RR intensities of charge-transfer systems.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as a result of this application.

Oct. 15, 1979
Date

Ronald H. Felton
Principal Investigator